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14. ABSTRACT

Breast cancer continues to be the most common malignancy affecting women. Although great strides have been made in the treatment and cure of early stage breast cancer, metastatic breast cancer remains incurable resulting in 40,000 deaths per year in the United States. In the setting of early stage disease, after completing definitive adjuvant treatment, there is no good marker of disease recurrence except once apparent from clinical symptoms or radiographically. At that point, the disease is no longer curable. Tumor markers currently in use are not able to distinguish potentially curable disease recurrence early enough to therapeutically intervene and thus avoid incurable disease. Circulating tumor cells are currently under investigation as a potential surrogate marker of such high risk situations, but there are no conclusive data. Two isoforms of proliferating cell nuclear antigen (PCNA) have been identified. The acidic isoform has been associated with neoplasia and can be identified by a specific antibody, demonstrated to distinguish between normal breast tissue and invasive breast cancer by immunohistochemistry. We propose to utilize this newly identified antibody to detect the cancer associated form of PCNA from both normal volunteers and breast cancer patients to evaluate whether there is a significant detectable difference.

15. SUBJECT TERMS

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INTRODUCTION

Two separate isoforms of the marker of cellular proliferation, proliferating cell nuclear antigen (PCNA) have recently been identified[3]. The acidic isoform, as opposed to its basic counterpart has been associated with neoplasia. This cancer associated PCNA (caPCNA) can now be identified by a specific antibody, caPCNAab, demonstrated to distinguish between normal breast tissue and invasive breast cancer by IHC. Additionally, this rabbit polyclonal antibody can also detect circulating caPCNA in the serum of patients with breast cancer (unpublished data). These data suggest that caPCNA may be a potential biomarker of breast cancer and the antibody to caPCNA, caPCNAab, may serve as a detector of breast cancer. The clinical utility of this antibody to cancer specific PCNA is untested. We have proposed to utilize this newly identified antibody, in a recently created ELISA format, to distinguish the cancer associated form of PCNA (caPCNA) from the blood of both normal volunteers and breast cancer patients to evaluate whether there is a statistically significant detectable difference.

BODY

We have proposed a first evaluation of a potential new serum biomarker, caPCNA for breast cancer. Utilizing this newly identified antibody, in a recently created ELISA format, to detect the cancer associated form of PCNA (caPCNA) from the blood of both normal volunteers and breast cancer patients, we hope to be able to evaluate whether there is a statistically significant detectable difference.

- **Utilizing the caPCNAab, the antibody to caPCNA, to determine the feasibility of testing for the presence of detectable caPCNA from sera by ELISA testing.**
- **To characterize the distribution of caPCNA in normal controls, early stage and metastatic disease.**
- **Utilizing the caPCNAab, to evaluate the reproducibility of sera testing of caPCNA levels by ELISA testing in a normal cohort of subjects and in those with breast cancer.**

We will assess blood samples from 65 normal female controls, 65 pre-treatment patients (early stage breast cancer patients [Stages I, II, III] shortly after diagnosis and prior to definitive breast surgery, radiotherapy, and systemic therapy as appropriate), and 65 patients with metastatic breast cancer, for a total of 195 blood samples. All samples will be obtained de-identified from an established Biospecimen Repository at our institute. No patients need be recruited. Our main objective in this study is to determine if the fraction of breast cancer patients with elevated caPCNA in their sera exceeds that of normal controls. We will also make two additional comparisons: pre-treatment to normal controls, and pre-treatment to metastatic. We expect to have at least 61 evaluable sera in each of the three groups. Utilizing ELISA methodology with the caPCNAab, caPCNA serum levels will be determined for each sample. This study will provide the preliminary

data to pursue prospectively collected data for longitudinal analysis that if validated, may provide the tool to intervene and potentially cure recurrent disease if detected early enough.

The ELISA methodology for the caPCNAab has been further developed to be able to reliably use smaller amounts of sera/fluid for testing. The original ELISA assay had a lower limit of detection of ~10 ng purified caPCNA/assay well, while the newly optimized assay can detect as little as 20 pg of purified caPCNA per 100 ul added to an assay well. ELISA testing, using the original format, did not reliably detect circulating levels of caPCNA (~14 of 92 patient samples examined appeared to express caPCNA). ELISA testing in duplicate completed on all 195 sera samples as planned, demonstrated feasibility of testing patient sera, but did not demonstrate a statistically significant reaction that could clearly distinguish healthy control specimens from serum specimens derived from either pre-treatment breast cancer patients, or patients with recurrent disease. (5, 8 and 6 patients, respectively, had OD values in excess of 0.25 OD above background. Conversely 49 pts in the control group exhibited OD readings of 0.15 or less, while 45 patients in the primary cancer group and 40 patients in the recurrent disease group exhibited OD readings of 0.15 or less.

There was no difference between the controls, pre-treatment patients or the patients with metastatic breast cancer. The first test, the Kruskal-Wallace, was an overall assessment of the three groups indicating that they do not differ, p-value =0.405. Figure 1 (see appendix) shows the cumulative distributions of caPCNA for the three groups. We also applied the Wilcoxon 2 sample test to each pair of groups and found no pair to significantly differ:

a. Control (n=64) vs pre-treatment (n=64), one sided p = 0.300
b. Control (n=64) vs metastatic (n=67), one sided p = 0. 485
c. Pre-treatment (n=64) vs metastatic (n=67), one sided p = 0.284

KEY RESEARCH ACCOMPLISHMENTS

- Optimized the ELISA methodology for the caPCNAab testing.
- ELISA testing in duplicate completed on all 195 sera samples as planned.

REPORTABLE OUTCOMES

None.

CONCLUSIONS

We have developed a reliable ELISA assay that is capable of consistently detecting circulating levels of caPCNA in the human sera. We demonstrated that our anti-caPCNA

antibody selectively captured caPCNA, and that binding to caPCNA could be competed against by either peptide antigen used to raise the antibody or pre-incubation of the interaction domain of a caPCNA binding partner with the sera containing caPCNA. We also further optimized this assay to detect as little as 20 pg of caPCNA in 100 µl of serum. While the hope was to be able to utilize the ELISA to distinguish patients who did not have cancer, (or those who were currently disease-free), from those with recurrent disease, our data indicates that all three groups appear to express an equivalent range of concentrations of caPCNA across the sample population tested.

One possibility for this observation is that small differences in the amount of circulating caPCNA between the groups could be masked by the high background of our current assay. Closer inspection of the raw data indicated that the assay background levels associated with the formation of the colored reaction product (nitrophenol) was artificially high, and seemed to be, at least in part, related to the specific type of commercially available general anti-PCNA primary antibody and anti-species specific alkaline phosphatase conjugated secondary antibody used in our assay. Thus, efforts to reduce the artificially high background are proceeding on two fronts. In the first approach, we are searching for better/different primary and secondary antibodies to see if a new combination of these (from alternate suppliers) helps reduce the intensity of the background control reactions. Our second approach, substitutes Horse Radish Peroxidase (HRP) as the secondary antibody conjugate in place of Alkalaine Phosphatase (AP). Should utilization of HRP not measurably improve background intensity of the negative control reactions, we will substitute a fluorescent tag such as Fluorescene or 5-methyl-FAM, for the AP/HRP, with the goal of increasing the signal to noise ratio of the assay, and decreasing the amount of caPCNA needed for detection. Both approaches have the potential to improve the sensitivity of the assay, thereby potentially distinguishing patients with either untreated or recurrent disease from healthy controls.

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None.

LIST OF PERSONNEL

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Samuel Litwin, Ph.D., Biostatistician

APPENDICES

Figure 1. Cumulative distributions of caPCNA.

Figure 1. Cumulative Distributions of caPCNA. ELISA testing on all 195 samples in duplicate as below did not demonstrate a statistically significant difference between normal healthy controls, those with primary untreated breast cancer and those with recurrent, metastatic breast cancer, Kruskal-Wallace test p-value = 0.405.